The Ras signaling pathway in *Drosophila*

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During *Drosophila* eye development, a Ras cascade mediates the decision between neuronal and non-neuronal differentiation of the R7 photoreceptor precursor. Recent genetic and molecular studies have identified a set of protein kinases as components of the Ras cascade and nuclear targets of the cascade, including Yan, Pointed, Jun, and Phyllopod. The Ras cascade functions in other *Drosophila* signal transduction pathways, eliciting a distinct response in each case, presumably through phosphorylation of specific transcription factors.

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Introduction

Ras is a key component of signal transduction pathways initiated by receptor tyrosine kinases (RTKs). Genetic studies of RTK pathways in *Drosophila melanogaster* and *Caenorhabditis elegans* (see the review in this issue by PS Kayne and PW Sternberg [pp 38–43]) and biochemical studies in mammalian systems (see the review in this issue by F McCormick [pp 51–55]) support this premise and further suggest that a set of factors functioning upstream and downstream of Ras is shared by RTKs, collectively forming a 'Ras cascade'. The Ras cascade transfers a signal from an activated RTK at the plasma membrane through the cytoplasm and into the nucleus, where it regulates the expression and/or the function of genes required to carry out the instructions of the initial signal.

Here, we will review recent progress toward understanding the role of *Ras1*, the *Drosophila* homolog of the transforming genes Ha-*ras*, Ki-*ras*, and N-*ras*, in eye development [1]. Particular attention will be paid to the role of Ras1 in a signal transduction pathway that determines the fate of a single cell type in the eye. Current evidence suggests that this pathway may serve as a paradigm for Ras function in RTK pathways in *Drosophila* and other organisms.

Eye development: an overview

Ras1 is required for the development of all photoreceptors in the *Drosophila* eye [2]. The *Drosophila* compound eye is composed of approximately 800 identical units called ommatidia, each of which contains eight photoreceptor cells (R1–R8), four non-neuronal cone cells, and eight accessory cells arranged in a highly ordered pattern. In third instar larvae, this pattern develops from an undifferentiated field of pluripotent cells, the eye imaginal disc (reviewed in [3]). The establishment of cellular identity in the developing eye disc is independent of cell lineage; instead, it occurs through a series of cell–cell interactions by which undifferentiated cells receive signals from neighboring cells, inducing them to adopt a specific fate.

Ommatidial assembly is initiated by neuronal differentiation of the R8 precursor cell, followed by R2/R5, R3/R4, R1/R6, and R7 precursors. Addition of cone cells and accessory cells to the photoreceptor cluster produces the final ommatidial complement. Cells within ommatidia can be identified on the basis of morphology and position. The 'outer' photoreceptors, R1–R6, have large rhabdomeres, the light-sensitive apparatus, and surround the 'inner' photoreceptors, R7 and R8, which have smaller rhabdomeres.

From a mechanistic standpoint, the process of photoreceptor recruitment probably occurs in two steps, each directed by signals transmitted via cell–cell interactions (Fig. 1). First, a particular undifferentiated cell is chosen to enter the developing ommatidium and is instructed whether or not to undergo neuronal differentiation. Second, if a neuronal pathway is chosen, the fate is narrowed down to a specific type of neuron, R1–R8. It should be noted that neuronal sub-type specification (i.e. step 2) may occur before neuronal differentiation (i.e. step 1). Currently, no data are available that distinguish the order of these steps.

Abbreviations

Boss—Bride-of-sevenless; **Drk**—Downstream of receptor kinase; **Egfr**—epidermal growth factor receptor; ***Elp***—*Ellipse*; **MAPK**—mitogen-activated protein kinase; **MAPKK**—mitogen-activated protein kinase kinase; **MAPKKK**—mitogen-activated protein kinase kinase kinase; **R**—photoreceptor cell; **RTK**—receptor tyrosine kinase; **Sev**—Sevenless; **SH**—Src homology; **Sina**—Seven-in-absentia; **Sos**—Son-of sevenless; **Tor**—Torso.

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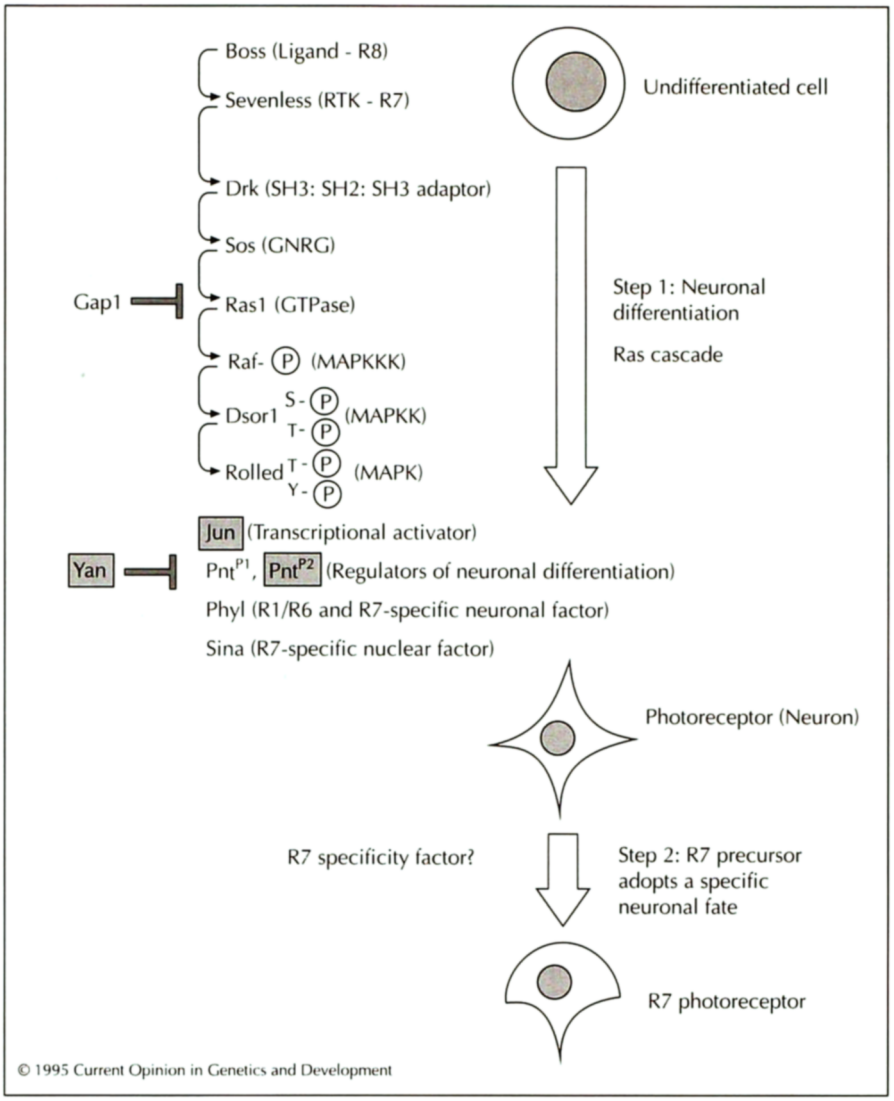

Fig. 1. Molecular and cellular events that occur during R7 differentiation. The Sev pathway is divided into two steps. Step 1, neuronal differentiation of the R7 precursor cell, is the focus of this review and consists of a Ras cascade and a set of downstream nuclear factors. Step 2, neuronal sub-type specification, is directed by distinct nuclear factors in the case of outer photoreceptors; however, an R7 specificity factor has yet to be identified. The Ras cascade is represented by a large open arrow. A list of Ras cascade components on the left are shown in the order in which they function, illustrated by arrows. Phosphorylated residues are indicated for Raf, Dsor1, and Rolled [20]. Downstream transcription factors that are phosphorylated by MAPK are indicated by tinted boxes. Gap1 and Yan are negative regulators of the pathway (shown as darkly tinted bars).

The Sevenless receptor tyrosine kinase pathway

Neuronal differentiation of the R7 cell is the best understood event in ommatidial assembly, as a result of numerous genetic screens that have identified genes involved in R7 production (Fig. 1). For simplicity and brevity, we will summarize much of this work (which has been reviewed elsewhere [4,5]). R7 differentiation is initiated by an interaction between the transmembrane Sevenless (Sev) RTK, expressed in the R7 precursor cell, and the Bride-of-sevenless (Boss) ligand, expressed exclusively in the R8 cell. The union of Sev and Boss leads to internalization of Boss by the R7 precursor cell and Sev activation by autophosphorylation.

Sev activation initiates a signal transduction cascade that is mediated by Ras1 (Fig. 1). Ras proteins function as signal transducers by cycling between inactive GDP-bound and active GTP-bound forms [6]. Son-of-sevenless (Sos) is a guanine nucleotide-releasing factor that activates Ras1 by promoting the exchange of GDP for GTP [2,7], and Gap1, a GTPase-activating protein, inactivates Ras1 by stimulating its intrinsic GTPase activity [8]. Loss-of-function mutations in *Gap1* [8] or expression of an activated form of Ras1 [9] under control of the *sev* promoter and enhancer, *sev-Ras1*^{Val12}, activates the Ras cascade in non-neuronal cone cells, transforming them into R7 cells. This disrupts the normal ommatidial array and produces an externally visible rough eye. Ectopic R7 specification in these backgrounds is independent of *sev*, suggesting that Ras1 activation is sufficient to promote neuronal differentiation in R7 and cone cell precursors.

A physical link between Ras1 regulatory factors and the Sev RTK is provided by Downstream of receptor kinase (Drk), which binds Sos and autophosphorylated Sev through its Src homology (SH) domains [10•,11•]. The Sev/Drk/Sos complex activates Ras1, which presumably then binds Raf, the *Drosophila* homolog of the c-Raf serine/threonine kinase [12]. Recent reports in mammalian systems suggest that the sole function of Ras, with respect to Raf, is to recruit Raf to the plasma membrane, where it is activated by an unknown mechanism, pos-

sibly phosphorylation [13,14]. In agreement with this hypothesis, membrane localization of *Drosophila* Raf, artificially achieved by fusing the Raf kinase domain to the Torso (Tor) RTK transmembrane and extracellular domains, sE-raf^{tor}, results in an activated Raf that bypasses the requirement for Ras1 in R7 determination [15].

Raf, a mitogen-activated protein kinase kinase kinase (MAPKKK), phosphorylates the Dsor1 tyrosine/threonine kinase, a mitogen-activated protein kinase kinase (MAPKK) [16•,17], which ultimately phosphorylates the Rolled serine/threonine kinase, a mitogen-activated protein kinase (MAPK) [18,19•]. Although no biochemical evidence exists for this chain of events in *Drosophila*, data from other systems are sufficient to suggest that the *Drosophila* Ras cascade functions similarly [20], and by genetic criteria, through analysis of double mutant strains, these genes have been shown to function in this order (Fig. 1) [15,16•,19•]; for example, loss-of-function mutations in *rolled* were identified as dominant suppressors of the rough eye phenotype produced by constitutively active Ras1 or Raf (sev-Ras1^{Val12} or sE-raf^{tor}, respectively), indicating that *rolled* is required for R7 development and that it acts downstream of *Ras1* and *raf* [19•,21].

Like Drk, Sos, Ras1 [2], and Raf [15], Rolled is probably required for development of additional photoreceptors, as flies homozygous for a weak allele of *rolled* contain fewer photoreceptor cells per ommatidium [19•]. A gain-of-function allele of *rolled*, *rolled*^{Sevenmaker}, resulting from a single amino acid change within the kinase domain, dominantly transforms cone cells into R7 cells in the absence of Sev activation, demonstrating that activation of Rolled is both necessary and sufficient to stimulate the Sev pathway and promote neuronal differentiation (step 1 in Fig. 1) [22••].

Rolled/MAPK targets

Activated Rolled/MAPK is thought to propagate the Ras cascade signal by phosphorylating a series of targets that presumably control expression and/or function of downstream genes required to execute the R7 developmental fate. MAPK proteins have been shown to phosphorylate serine and threonine residues within the consensus recognition site Pro-X-Ser/Thr-Pro (in the three-letter amino acid code, where X can be any amino acid) [20]. Genetic dissection of R7 cell determination has begun to shed light on the events downstream of Rolled/MAPK.

It has been shown recently that two members of the Ets family of transcription factors, Yan and Pointed, function in the Sev pathway and are targets for phosphorylation by Rolled (Fig. 1) [23••,24•]. Flies homozygous for a hypomorphic *yan* mutation have extra R7 and outer photoreceptor cells, even in the absence of *sev* function [25,26], and loss-of-function *yan* mutations dominantly enhance the rough eye phenotypes of *Gap1*, *sev-Ras1*^{Val12}, and *sE-raf*^{tor}, suggesting that it functions as a negative regulator of the Sev pathway [21,25]. In contrast, *pointed* mutations suppress *Gap1* and *sev-Ras1*^{Val12} induced rough eye phenotypes, suggesting that it acts as a positive regulator of the Sev pathway [23••].

The *pointed* gene is transcribed from two promoters, P1 and P2, which produce transcripts encoding two related proteins that are expressed in all cells prior to neuronal differentiation [23••,24•,27]. The Pointed^{P2} protein contains a single MAPK phosphorylation site, and *in vitro* kinase assays have shown that it can be phosphorylated by MAPK and that this phosphorylation is dependent on the MAPK site [24•]. Co-transfection experiments in *Drosophila* tissue culture cells have demonstrated that activation of the Ras cascade stimulates the ability of Pointed^{P2} to activate transcription, and that this activity is also dependent on the presence of the phospho-acceptor residue [23••]. Therefore, these results suggest that Pointed^{P2} function is stimulated by Rolled/MAPK phosphorylation.

Yan contains eight consensus MAPK phosphorylation sites, at least one of which is phosphorylated in response to activation of the Ras cascade in tissue culture cells [23••]. In the co-transfection assays described above, Yan was shown to repress transcriptional activation by Pointed^{P1}. This repression is alleviated by activation of the Ras cascade, suggesting that Rolled/MAPK phosphorylation of Yan reduces its ability to repress transcription. Different scenarios can explain this observation. The simplest one would be that Pointed and Yan compete for the same DNA-binding site(s) or protein partner(s) and that a Rolled/MAPK-phosphorylated Yan would have reduced affinity for its target(s), thus allowing Pointed to activate transcription. Alternatively, phosphorylation could induce a destabilization or a relocalization of Yan. It is also possible that Yan functions as a positive regulator of transcription whose target genes would antagonize Pointed-mediated transcription. In this case, phosphorylation of Yan by Rolled/MAPK would abrogate its transcriptional properties.

Recently, another transcription factor, Jun, was implicated in the determination of photoreceptor fate [28••]. Jun is a member of the AP-1 family of transcription factors, which in mammalian cells function in growth control and regulation of differentiation [29]. Interestingly, the *Drosophila* homolog of Jun is expressed in a spatial and temporal pattern that matches photoreceptor differentiation, but its expression precedes the appearance of neuronal antigens. Expression of a dominant-negative form of Jun under control of the *sev* enhancer results in missing photoreceptors that correspond to cells in which the mutant protein is expressed. Furthermore, dominant-negative Jun suppresses the cone cell → R7 cell transformation triggered by constitutive activation of either Sev, Ras1, Raf, or Rolled. These results suggest that Jun functions downstream of the Ras cascade

in R7 cell determination (Fig. 1). Since the expression pattern of Jun is unaltered in a *sev* background, Sev activation of Jun is probably post-transcriptional. In fact, the kinase sites that are implicated in activation of human c-Jun by the Ras cascade are conserved in *Drosophila* Jun and may be targets for Rolled/MAPK or a *Drosophila* homolog of the recently identified Jun-specific kinase, JNK1 [30–32].

In screens for dominant suppressors of the extra R7 cell phenotype produced by *sev-Ras1*^{Val12} or *sE-raf*^{tor}, two research groups have independently identified a gene, *phyllopod*, which encodes a novel nuclear factor that functions downstream of the Ras cascade in the Sev pathway (Fig. 1) [33••,34••]. In the developing eye, *phyllopod* is expressed in a subset of photoreceptors, namely R1/R6 and R7, and is required in those cells for proper differentiation. Using the *sev* enhancer/promoter system, it has been shown that misexpression of *phyllopod* in cone cells converts them into R7-like cells, suggesting that Phyllopod can specify neuronal fate. In fact, ectopic expression of Phyllopod in accessory pigment cells, which do not ordinarily express Phyllopod, induces expression of neuronal antigens. It has thus been postulated that one role of the Ras cascade in the presumptive R7 cell is to induce *phyllopod* expression.

In agreement with multiple studies from mammalian systems, genetic studies in *Drosophila* have identified a connecting point between the Ras cascade and nuclear factors required for developmental decisions. This link is provided, at least in part, by Rolled/MAPK, which directly modulates, by phosphorylation, the activity of transcription factors involved in cell fate determination. In addition, the *Drosophila* model has allowed the identification of the novel gene, *phyllopod*, required for neuronal determination and the expression of which appears to depend on the Ras cascade. It will be interesting to verify whether *phyllopod* transcription is induced by Rolled/MAPK-modified transcription factors, and to investigate the possibility that the Phyllopod protein and its role in Ras-dependent neuronal differentiation have been conserved throughout evolution.

Other receptor tyrosine kinase pathways

In addition to Sev, the Ras cascade functions downstream of other *Drosophila* RTKs, including Tor [35,36], and *Drosophila* homologs of epidermal growth factor receptor (Egfr) [37] and fibroblast growth factor receptor (DFGF-R1/Breathless) [38]. Tor functions in the embryonic terminal system, which is responsible for specification of the tail and unsegmented head regions [39]. Loss-of-function *tor* mutations result in deletion of these terminal structures, whereas a gain-of-function mutation, *tor*^{RL3}, shows the opposite phenotype, an absence of segmentation in the middle region and an expansion of the terminal structures [39,40]. Loss-of-function *Egfr* mutations cause pleiotropic phenotypes affecting patterning of follicle cells during oogenesis, wing vein differentiation, and photoreceptor development, whereas a gain-of-function *Egfr* allele, *Ellipse* (*Elp*), causes a reduction in the number of ommatidia and additional veins in the wing [41,42]. Breathless is required during embryonic development for the migration of tracheal and midline glial cells [43].

Mutations in *drk*, *Sos*, *Ras1*, *raf*, and *rolled* dominantly suppress phenotypes produced by gain-of-function mutations of *sev* (*sev*^{S11}), *Egfr* (*Elp*), and *tor* (*tor*^{RL3}) [2,19•,21], and activated *Ras1* and *raf* suppress the *breathless* tracheal migration defect [38]. Furthermore, *rolled*^{Sevenmaker}, the gain-of-function *rolled* allele, produces phenotypes that mimic *sev*^{S11}, *Elp*, and *tor*^{RL3} [22••]. Therefore, it appears that the Ras cascade functions as a naive transmitter of all RTK signals. The identification of MAPK targets in these pathways may clarify how the Ras cascade produces such diverse responses.

A Ras cascade in other photoreceptors

Does a Ras cascade promote neuronal differentiation in photoreceptor precursor cells other than R7? This appears to be the case, as the development of all photoreceptors, not just R7, is affected in homozygous clones of loss-of-function mutations in *Ras1* or other components of the cascade [2,15,19•]. Furthermore, targets of Rolled/MAPK (i.e. Yan and Pointed^{P2}) are expressed in all undifferentiated cells in the eye disc and are required for neuronal differentiation of other photoreceptors in addition to R7 [23••,24•].

To date, specific RTKs, analogous to Sev, that activate the Ras cascade in R2/R5, R4/R4, R1/R6, or R8 have not been identified. Egfr may fulfill this role in some photoreceptor precursors, as it appears to be required for neuronal differentiation of all photoreceptors; however, this has been difficult to assess, as Egfr is required for both proliferation and differentiation during eye development [45].

Specification of photoreceptor sub-type

If the Ras cascade is a common inducer of neuronal differentiation in all photoreceptors, then specification of neuronal sub-type (i.e. R1–R8) must require another signal input (step 2 in Fig. 1). Presumably, the developmental history of a photoreceptor precursor cell directs the expression of genes that determine sub-type. The expectation is that loss-of-function mutations in these determination genes will affect sub-type specification and not neuronal differentiation. Two genes, *rough* and *seven-up*, fit this model.

Rough is a homeodomain protein that is expressed in R2/R5 and R3/R4, and it appears to specify the R2/R5 outer photoreceptor cell fate [46,47]. In loss-of-function *rough* mutations, cells that would normally develop as R2/R5 are transformed toward the R3/R4/R1/R6 fate [48]. The ability of Rough to specify outer photoreceptor fate is demonstrated most clearly by ectopic expression of *rough* using the *sev* enhancer/promoter system, which results in transformation of the R7 precursor cell into an outer, R1–R6-like, photoreceptor neuron [46,49]. Seven-up is a member of the steroid receptor superfamily that is expressed and required in R3/R4 and R1/R6 for normal ommatidial development [50]. In the absence of Seven-up, these precursor cells are transformed to an R7 fate, suggesting that Seven-up specifies outer photoreceptor fate in R3/R4 and R1/R6.

An additional nuclear protein that fulfills some of the criteria for a specificity factor is Seven-in-absentia (Sina). Sina is expressed in many cells in the developing eye, but it is required only in the R7 precursor for normal cell-fate specification [51]. In the absence of *sina*, the presumptive R7 cell fails to express neuronal antigens and adopts the fate of a non-neuronal cone cell. Thus, loss-of-function mutations in *sina*, like *rough* and *seven-up*, alter cell-type specification, but, unlike *rough* and *seven-up*, *sina* mutations prevent neuronal differentiation.

Conclusions and future directions

Genetic dissection of the Sev RTK signal transduction pathway has led to the identification of a Ras cascade, the function of which is to promote neuronal differentiation of photoreceptor precursor cells. The Ras cascade functions downstream of other RTKs in *Drosophila*, eliciting different responses in each case, presumably through regulation of specific transcription factors.

Several issues concerning the Ras cascade remain to be resolved. First, how is Raf activated? The interaction of Raf with Ras and a newly identified family of proteins, 14-3-3 proteins, may be sufficient to stimulate its activity, but it is possible that activation requires additional factors, such as a kinase that phosphorylates Raf [52,53]. Second, are there negative regulators of the Ras cascade, other than Gap1? Obvious candidates are protein phosphatases, which could inactivate components of the cascade that are positively regulated by phosphorylation. Phosphatases that are specific for MAPK have been identified in mammalian systems — CL100 and PAC1 in humans and 3CH134 in mouse [54]. These dual-specificity phosphatases catalyze dephosphorylation of both tyrosine and serine/threonine residues. Although enzymes of this class have not been identified in *Drosophila*, serine/threonine phosphatases (such as protein phosphatase 2A) which dephosphorylate MAPK *in vitro* have been identified. Third, what are the gene targets for transcription factors regulated by the Ras cascade, and are additional factors regulated by the Ras cascade? Further genetic analysis of RTK pathways in *Drosophila* may provide answers to these questions.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
- • of special interest
- •• of outstanding interest

1. Neumann-Silberberg FS, Schejter E, Hoffmann FM, Shilo B: **The *Drosophila* ras oncogenes: structure and nucleotide sequence.** *Cell* 1984, **37**:1027–1033.

2. Simon MA, Bowtell DDL, Dodson GS, Laverty TR, Rubin GM: **Ras1 and a putative guanine nucleotide exchange factor performs crucial steps in signaling by the Sevenless tyrosine kinase.** *Cell* 1991, **67**:701–716.

3. Wolff T, Ready DF: **Pattern formation in the *Drosophila* retina.** In *The development of Drosophila melanogaster.* Edited by Bate M, Martinez-Arias A. Cold Spring Harbor: Cold Spring Harbor Press; 1993:1277–1325.

4. Dickson B, Hafen E: *Genetic dissection of eye development in Drosophila.* Edited by Bate M, Martinez-Arias A. Cold Spring Harbor: Cold Spring Harbor Press; 1993:1327–1362.

5. Zipursky SL, Rubin GM: **Determination of neuronal cell fate: lessons from the R7 neuron of *Drosophila*.** *Annu Rev Neurosci* 1994, **17**:373–397.

6. Boguski MS, McCormick F: **Proteins regulating Ras and its relatives.** *Nature* 1993, **366**:643–654.

7. Rogge RD, Karlovich CA, Banerjee U: **Genetic dissection of a neurodevelopmental pathway: *Son of sevenless* function downstream of the *sevenless* and EGF receptor tyrosine kinase.** *Cell* 1991, **64**:39–48.

8. Gaul U, Mardon G, Rubin GM: **A putative Ras GTPase activating protein acts as a negative regulator of signaling by the Sevenless receptor tyrosine kinase.** *Cell* 1992, **68**:1007–1019.

9. Fortini ME, Simon MA, Rubin GM: **Signalling by the Sevenless protein tyrosine kinase is mimicked by Ras1 activation.** *Nature* 1992, **355**:559–561.

10. • Simon MA, Dodson GS, Rubin GM: **An SH3-SH2-SH3 protein is required for p21^{Ras1} activation and binds to Sevenless and SOS proteins *in vitro*.** *Cell* 1993, **73**:169–177.
This study provides biochemical evidence that Drk, an SH3:SH2:SH3 protein, binds to Sev, an RTK, and Sos, a guanine nucleotide releasing factor, and genetic evidence that Drk functions upstream of Ras1 in the Sev pathway. (Also see [11•] annotation.)

11. • Olivier JP, Raabe T, Henkemeyer M, Dickson B, Mbamalu G, Margolis B, Schlessinger J, Hafen E, Pawson T: **A *Drosophila* SH2-SH3 adaptor protein implicated in coupling the Sevenless tyrosine kinase to an activator of Ras guanine nucleotide exchange, SOS.** *Cell* 1993, **73**:179–191.

Drk, the *Drosophila* homolog of *C. elegans* Sem-5 and human GRB2, was shown to bind autophosphorylated RTKs with its SH2 domain and Sos through its SH3 domains. Futhermore, its activity was shown to correlate with localization to the plasma membrane. (Also see [10•] annotation.)

12. Moodie SA, Wolfman: **The 3Rs of life: Ras, Raf, and growth regulation.** *Trends Genet* 1994, **10**:44–48.

13. Stokoe D, Macdonald SG, Cadwaller K, Symons M, Hancock JF: **Activation of Raf as a result of recruitment to the plasma membrane.** *Science* 1994, **264**:1463–1467.

14. Leevers SJ, Paterson HF, Marshall CJ: **Requirement for Ras and Raf activation is overcome by targeting Raf to the plasma membrane.** *Nature* 1994, **369**:411–414.

15. Dickson B, Sprenger F, Morrison D, Hafen E: **Raf functions downstream of Ras1 in the Sevenless signal transduction pathway.** *Nature* 1992, **360**:600–603.

16. • Tsuda L, Inoue YH, Yoo MA, Mizuno M, Hata M, Lim YM, Adachi-Yamada T, Ryo H, Masamune Y, Nishida Y: **A protein kinase similar to MAP kinase activator acts downstream of the Raf kinase in *Drosophila*.** *Cell* 1993, **72**:407–414.

Dsor1, a MAPKK, was shown to function downstream of Raf in the Tor pathway.

17. Lu X, Melnick MB, Hsu J-C, Perrimon N: **Genetic and molecular analyses of mutation involved in *Drosophila raf* signal transduction.** *EMBO J* 1994, **13**:2592–2599.

18. Biggs WH, Zipursky SL: **Primary structure, expression, and signal-dependent tyrosine phosphorylation of a *Drosophila* homolog of extracellular signal-regulated kinase.** *Proc Natl Acad Sci USA* 1992, **89**:6295–6299.

19. • Biggs WH, Zavitz KH, Dickson B, van der Straten A, Brunner D, Hafen E, Zipursky SL: **The *Drosophila rolled* locus encodes a MAP kinase required in the Sevenless signal transduction pathway.** *EMBO J* 1994, **13**:1628–1635.

Rolled, a MAPK, was shown to function downstream of Raf in the Sev pathway.

20. Marshall CJ: **MAP kinase kinase kinase, MAP kinase kinase and MAP kinase.** *Curr Opin Genet Dev* 1994, **4**:82–89.

21. Chang HC, Karim FD, O'Neill EM, Rebay I, Solomon NM, Therrien M, Wassarman DA, Wolff T, Rubin GM: **The Ras signal transduction pathway in *Drosophila* eye development.** *Cold Spring Harb Symp Quant Biol* 1995, in press.

22. •• Brunner D, Oellers N, Szabad J, Biggs WH, Zipursky SL, Hafen E: **A gain-of-function mutation in *Drosophila* MAP kinase activates multiple receptor tyrosine kinase signaling pathways.** *Cell* 1994, **76**:875–888.

The *rolled*^{Sevenmaker} gain-of-function allele of *rolled*/MAPK induces R7 development in the absence of Sev function, suggesting that activation of Rolled/MAPK is both necessary and sufficient for R7 induction. In addition, this mutation activates pathways initiated by the Tor and Egfr RTKs.

23. •• O'Neill EM, Rebay I, Tjian R, Rubin GM: **The activities of two Ets-related transcription factors required for *Drosophila* eye development are modulated by the Ras/MAPK pathway.** *Cell* 1994, **78**:137–147.

This paper and [24•] present the first genetic and biochemical demonstrations of specific MAPK targets in a *Drosophila* RTK pathway. In tissue culture assays, Pointed^{P1} is a constitutively strong transcriptional activator and is unresponsive to Ras1 activation, whereas Pointed^{P2} activity is stimulated by the Ras1/MAPK pathway. The activity of Pointed^{P2} is dependent on its single MAPK site. Yan, which is phosphorylated by MAPK in tissue culture cells, represses the activity of Pointed^{P1} and this repression is alleviated by Ras1/MAPK activation. This suggests that Yan and Pointed have antagonizing functions in the Sev pathway that are regulated by MAPK phosphorylation.

24. • Brunner D, Ducker K, Oeliers N, Hafen E, Scholz H, Klambt C: **The ETS domain protein Pointed-P2 is a target of MAP kinase in the Sevenless signal transduction pathway.** *Nature* 1994, **370**:386–389.

This work reiterates and complements that presented in [23••] by demonstrating that Pointed^{P2} is phosphorylated by MAPK *in vitro*. Furthermore, mutating the phosphoacceptor site in Pointed^{P2} eliminates its ability to rescue the *pointed* phenotype in transgenic animals.

25. Lai ZC, Rubin GM: **Negative control of photoreceptor development in *Drosophila* by the product of the *yan* gene, an ETS domain protein.** *Cell* 1992, **70**:609–620.

26. Tei H, Nihonmatsu I, Yokokura T, Ueda R, Sano Y, Okuda T, Sata K, Hirata K, Fujita SC, Yamamoto D: ***pokkuri*, a *Drosophila* gene encoding an E-26-specific (Ets) domain protein, prevents overproduction of the R7 photoreceptor.** *Proc Natl Acad Sci USA* 1992, **89**:6856–6860.

27. Klambt C: **The *Drosophila* gene *pointed* encodes two Ets-like proteins which are involved in the development of the midline glial cells.** *Development* 1993, **117**:163–176.

28. •• Bohmann D, Ellis MC, Staszewski LM, Mlodzik M: ***Drosophila* Jun mediates Ras-dependent photoreceptor determination.** *Cell* 1994, **78**:973–986.

This study demonstrates genetically, as anticipated from mammalian studies, that in *Drosophila*, Jun functions downstream of the Ras cascade. Jun appears to be required for the development of all photoreceptor cells, acting downstream of Ras1 to mediate the decision between neuronal and non-neuronal cell fates.

29. Vogt PK, Bos TJ: **jun: oncogene and transcription factor.** *Adv Cancer Res* 1990, **55**:1–35.

30. Binetruy B, Smeal T, Karin M: **Ha-ras augments c-Jun activity and stimulates phosphorylation of its activation domain.** *Nature* 1991, **351**:122–127.

31. Pulverer BJ, Kyriakis JM, Aruch J, Nikolakaki E, Woodgett JR: **Phosphorylation of c-Jun mediated by MAP kinase.** *Nature* 1991, **353**:670–674.

32. Derijard B, Hibi M, Wu I, Barrett T, Su B, Deng T, Karin M, Davis RJ: **JNK1: a protein kinase stimulated by UV-light and Ha-Ras that binds and phosphorylates the c-Jun activation domain.** *Cell* 1994, **76**:1025–1037.

33. •• Chang HC, Solomon NM, Wassarman DA, Karim FD, Therrien M, Rubin GM, Wolff T: ***phyllopod* functions downstream of *Ras1* in the fate determination of a subset of photoreceptors in *Drosophila*.** *Cell* 1995, in press.

See [34••] annotation.

34. •• Dickson BJ, Dominguez M, van der Straten A, Hafen E: **Control of *Drosophila* photoreceptor fates by Phyllopod, a novel nuclear protein acting downstream of Raf.** *Cell* 1995, in press.

This paper and [33••] provide compelling evidence that *phyllopod* is a photoreceptor-specific target of the Ras cascade. Phyllopod expression appears to depend on activation of the Ras cascade, and its activity is required in R1/R6 and R7 precursor cells for their neuronal specification.

35. Doyle HJ, Bishop JM: **Torso, a receptor tyrosine kinase required for embryonic pattern formation, shares substrates with the Sevenless and EGF-R pathways in *Drosophila*.** *Genes Dev* 1993, **7**:633–646.

36. Hsu J-C, Perrimon N: **A temperature-sensitive MEK mutation demonstrates the conservation of the signaling pathways activated by receptor tyrosine kinases.** *Genes Dev* 1994, **8**:2176–2187.

37. Diaz-Benjumea FJ, Hafen E: **The Sevenless signalling cassette mediates *Drosophila* EGF receptor function during epidermal development.** *Development* 1994, **120**:569–578.

38. Reichman-Freid M, Dickson B, Hafen E, Shilo BZ: **Elucidation of the role of *breathless*, a *Drosophila* FGF receptor homolog, in tracheal cell migration.** *Genes Dev* 1994, **8**:428–439.

39. Perrimon N: **The Torso receptor protein-tyrosine kinase signaling pathway: an endless story.** *Cell* 1993, **74**:219–222.

40. Klingler M, Erdelyi M, Szabad J, Nusslein-Volhard C: **Function of torso in determining the terminal anlagen of the *Drosophila* embryo.** *Nature* 1988, **335**:275–277.

41. Shilo BZ, Raz E: **Developmental control by the *Drosophila* EGF receptor homolog DER.** *Trends Genet* 1991, **7**:388–392.

42. Baker NE, Rubin GM: **Effect on eye development of dominant mutations in *Drosophila* homologue of the EGF receptor.** *Nature* 1989, **340**:150–153.

43. Klambt C, Glazer L, Shilo BZ: ***breathless*, a *Drosophila* FGF receptor homolog is essential for migration of tracheal and specific midline glial cells.** *Genes Dev* 1992, **6**:1668–1672.

44. Basler K, Christen B, Hafen E: **Ligand-independent activation of the Sevenless receptor tyrosine kinase changes the fate of cells in the developing *Drosophila* eye.** *Cell* 1991, **64**:1069–1081.

45. Xu T, Rubin GM: **Analysis of genetic mosiacs in developing and adult *Drosophila* tissues.** *Development* 1993, **117**:1223–1237.

46. Kimmel BE, Heberlein U, Rubin GM: **The homeodomain protein rough is expressed in a subset of cells in the developing *Drosophila* eye where it can specify photoreceptor cell subtype.** *Genes Dev* 1990, **4**:712–727.

47. Tomlinson A, Kimmel BE, Rubin GM: ***rough*, a *Drosophila* homeobox gene required in photoreceptors R2 and R5 for inductive interactions in the developing eye.** *Cell* 1988, **55**:771–784.

48. Heberlein U, Mlodzik M, Rubin GM: **Cell-fate determination in the developing *Drosophila* eye: role of the *rough* gene.** *Development* 1991, **112**:703–712.

49. Basler K, Yen D, Tomlinson A, Hafen E: **Reprogramming cell fate in the developing *Drosophila* retina: transformation of R7 cells by ectopic expression of *rough*.** *Genes Dev* 1990, **4**:728–739.

50. Mlodzik M, Hiromi Y, Weber U, Goodman CS, Rubin GM: **The *Drosophila seven-up* gene, a member of the steriod receptor gene superfamily, controls photoreceptor cell fates.** *Cell* 1990, **60**:211–224.

51. Carthew RW, Rubin GM: ***seven-in-absentia*, a gene required for specification of R7 cell fate in the *Drosophila* eye.** *Cell* 1990, **63**:561–577.

52. Freed E, Symons M, Macdonald SG, McCormick F, Ruggieri R: **Binding of 14-3-3 proteins to the protein kinase Raf and effects on its activation.** *Science* 1994, **265**:1713–1716.

53. Irie K, Gotoh Y, Yashar BM, Errede B, Nishida E, Matsumoto K: **Stimulatory effects of yeast and mammalian 14-3-3 proteins on the Raf protein kinase.** *Science* 1994, **265**:1716–1719.

54. Nebreda AR: **Inactivation of MAP kinases.** *Trends Biochem* 1994, **19**:1–2.

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